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Review

COVID-19 associated cardiac disease: Is there a role of neutrophil extracellular traps in pathogenesis?

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Abstract: The COVID-19 pandemic has driven an upheaval of new research, providing key insights into the pathogenesis of this disease. Lymphocytopenia, hyper-inflammation and cardiac involvement are prominent features of the disease and have prognostic value. However, the mechanistic links among these phenomena are not well understood. Likewise, some COVID-19 patients exhibit multi-organ failure with diseases affecting the cardiac system, appearing to be an emerging feature of the COVID-19 pandemic. Neutrophil extracellular traps (NETs) have been frequently correlated with larger infarct sizes and can predict major adverse cardiac events. However, the exact mechanism behind this remains unknown. Although the excessive NET formation can drive inflammation, particularly endothelial and promote thrombosis, it is essential to normal immunity. In this paper, we postulate the role of NETs in cardiac disease by providing an overview of the relationship between NET and inflammasome activities in lung and liver diseases, speculating a link between these entities in cardiac diseases as well. Future research is required to specify the role of NETs in COVID-19, since this carries potential therapeutic significance, as inhibition of NETosis could alleviate symptoms of this disease. Knowledge gained from this could serve to inform the assessment and therapeutics of other hyper inflammatory diseases affecting the heart and vasculature alike.

Keywords: COVID-19; SARS-CoV2; neutrophil extracellular traps (NETs); cardiovascular disease; angiotensin-converting enzyme 2 (ACE2) receptor; multi-organ failure

1. Introduction

The global pandemic of SARS-CoV-2 mediated coronavirus disease-2019 (COVID-19) has strongly impacted the scope of health systems worldwide, most frequently manifesting as acute respiratory distress syndrome (ARDS) characterized by a systemic inflammatory response syndrome (SIRS) and multi-organ failure. Several cardiac manifestations have been well documented in COVID-19, including myocardial injury, acute coronary syndrome (ACS), cardiomyopathy, arrhythmias, and cardiogenic shock, as well as multiple venous thromboembolic complications, including DIC (Disseminated Intravascular Coagulation) and DVT [1]. Such manifestations are common, with myocardial injury and concurrently elevated cardiac enzymes above the 99th percentile occurring in 20–30% of hospitalized COVID-19 patients [2]. Moreover, elevated cardiac biomarkers in patients with COVID-19 positively correlate with disease severity [3,4]. To this extent, cardiac diseases are an emerging feature of COVID-19 infections, and myocardial injury bears a significant contribution to a fatal outcome [4]. However, the mechanisms underlying these findings remain to be elucidated.

Neutrophil extracellular traps (NETs) have been implicated in various cardiac pathologies, such as ST-Elevation Myocardial Infarction (STEMI), Acute Coronary Syndrome (ACS), and coronary artery disease. NETs are DNA-based fibrous structures released by neutrophils upon stimulation by pro-inflammatory cytokines and directly by various immune cells. NETs are physiologically essential in controlling microbial infections, however excessive NET secretion induces collateral damage to nearby host cells through various cytotoxic elements, propagating inflammation [5,6]. Since neutrophilia is a cardinal feature of severe COVID-19 infections [7–9], we highlight NETs as playing central roles in the pathogenesis of COVID-19, with an emphasis on its cardiac manifestations.

2. Pathophysiological mechanisms underlying Covid-19

The mechanistic pathways underlying these systemic, multiorgan manifestations of COVID-19 have garnered much interest. Multiple studies have described and analyzed the longitudinal changes in circulating immune cells throughout the COVID-19 disease course and compared the serum profiles of proinflammatory cytokines in varying severities of disease [3,10]. Correspondingly, proinflammatory signatures of severely affected COVID-19 patients feature sharp elevations of IL-1β, IL-6, and TNF-α [11], which activate neutrophils. This suggests an impairment of adaptive antiviral immune responses, with a concurrent amplification of the innate response mediated by neutrophils [12–14], leading to widespread microvascular thrombosis. This is supported by postmortem histopathological studies reporting that the diverse clinical manifestations of COVID-19 result from diffuse alveolar damage with thrombotic microangiopathy, which is characterized by diffuse microvascular platelet-fibrin thrombi adjacent to necrotic areas in pulmonary alveolar capillaries [15]. This also occurs in several remote organs, including the liver, gastrointestinal tract, kidney, and, the focus of the present discussion, the heart [16,17].

It is confirmed that SARS-CoV-2 infection can induce neutrophilia [18–20] with concurrent lymphopenia predominantly affecting T lymphocyte subpopulations [21]. Indeed, an increased neutrophil-to-lymphocyte ratio is considered the most important independent risk factor for severe COVID-19. Intriguingly, immature neutrophils make a sizeable contribution to the pool of peripheral neutrophilia in the sera of COVID-19 patients and also correlate with disease severity. Thus, suggestions have been made that specifically delineating the immature neutrophil phenotypes characterizing the

elevated neutrophil count would constitute a reliable predictive marker of disease severity [22]. However, these immature granulocytes have not been observed to dominate the peripheral pool to any extent, and a lack of current consensus regarding the nomenclature for neutrophil developmental stages currently hampers such investigations.

2.1. Neutrophil responses during COVID-19

Studies have implicated neutrophils as key players in COVID-19 [23]. They represent the most abundant circulating immune cell types in the circulation and characterize acute inflammation by infiltrating tissues and contributing to pathogen clearance via phagocytosis and degranulation. Therefore, an increased neutrophil count and neutrophil activation are considered hallmarks of severe COVID-19 [1,2].

More recently, investigations have focused on the production of neutrophil extracellular traps (NETs) by effete interstitial neutrophils. NETs are composed of a network of cytosolic neutrophil proteins with antimicrobial properties embedded in a cell-free DNA (cfDNA)-based meshwork. These proteins include neutrophil elastase (NE), myeloperoxidase (MPO), and histones (cit-H3) [24]. Owing to their fibrous nature, NETs serve to trap pathogens, thereby preventing microbial dissemination while simultaneously sequestering microbes in areas where antimicrobial NET contents are concentrated. However, such proteins also lead to collateral host tissue injury and, therefore, if dysregulated, can increase damage-associated molecular patterns (DAMPs) to amplify non-homeostatic immune responses featuring hyperinflammation and thrombosis.

These findings instigated studies assessing potential NETs involvement in thrombosis caused by COVID-19 [12,25]. Importantly, NET markers (NE, MPO, cit-H3) correlate positively with COVID-19 disease severity [26,27]. COVID-19 patient sera induce NETosis in healthy, control-derived neutrophils, and retrieved neutrophils from COVID-19 patients exhibit higher NET-ting at baseline [28]. Moreover, histopathologic analysis demonstrates rich NET infiltration within microthrombi in the pulmonary [26], hepatic, renal, and cardiac microvasculature [22,29]. Immature neutrophils, which characterize severe COVID-19, display enhanced NET release, which may account for the elevated NET markers in this setting. This is confirmed by a study demonstrating SARS-CoV-2 as directly stimulating NETosis, with infection being dependent on AE2 expression levels, through a pathway involving peptidyl arginine deaminase (PAD4) in COVID-19 patients [30]. These findings have resulted in currently ongoing clinical trials evaluating pharmacologic NET inhibition or lysis as potential treatments of COVID-19, which discussed in detail below.

2.2. Mechanism of NET formation

Neutrophil extracellular traps (NETs) formation can be split into two pathways broadly, a lytic and nonlytic path. Interestingly, NET formation from neutrophils can be directly induced by the spike (S) and nucleocapsid (N) proteins of SARS-CoV-2 [31]. Depending on the initiating stimulus providing the excitatory signal, either the lytic or non-lytic mechanism can be utilized [29,32,33].

Lytic NETosis: When neutrophils are stimulated along this pathway, reactive oxygen species (ROS) are produced, and nicotinamide adenine dinucleotide phosphate (NADPH) is activated. Pathways independent of NADPH oxidase facilitate the release of ROS from mitochondria. PAD4 activation

follows, which in turn converts arginine to citrulline on histone proteins, which removes the positive charges on arginine. Chromatin is then decondensed and a disarrangement of the electrostatic charges between histones and DNA takes place [34]. A spillover of decondensed chromatin and protein granule into the extracellular space occurs following nuclear membrane disintegration, resulting in loss of neutrophil viability.

Non-lytic NETosis: The binding of infectious stimuli to toll-like receptors (TLRs) and platelet-neutrophil engagement provide stimuli for driving this pathway [35]. Here, NADPH is not needed for PAD4 activation. Blebs of the nuclear envelope containing decondensed chromatin and embedded proteins are secreted and the nuclear envelope is sealed back [5,36]. The NETs released in these blebs are of varied compositions, housing either nuclear or mitochondrial DNA, with the latter providing physiologic protection as well. As a result, the viability of neutrophils is maintained in this pathway, with retention of vital functions like chemotaxis and phagocytosis [35].

2.3. NETs-mediated immuno-thrombosis

NETs are produced by a programmed form of neutrophilic death termed NETosis, leading to the extrusion of neutrophil contents such as DNA, histones, neutrophil granule proteins such as neutrophil elastase (NE), and neutrophil cytosolic proteins such as myeloid-related protein 14 (MRP14) [5]. NETs play key roles in immune defense through bactericidal and antimicrobial components such as histones, NE, and MRP14. NETs, due to their fibrous structure, sequester bacteria in areas where the concentration of its antimicrobial components is high while simultaneously preventing microbial dissemination [37].

Although NETs are physiologically essential in preventing microbial spread, their excessive production can drive inflammation via DAMPs and host cell injury, particularly of the endothelium, through histones [38,39]. Additionally, the fibrous structure of NETs serves as a nidus for the deposition of red blood cells (RBCs), platelets, fibrinogen, and fibronectin, thereby precipitating thrombus formation [40]. As such, many studies have implicated NETs to be underlying several disease processes, ranging from autoimmune conditions, e.g., systemic lupus erythematosus, atherosclerosis, to vascular diseases such as thrombosis, metabolic diseases including diabetes, and, the focus of this manuscript, cardiac disease, and COVID-19 [32].

NET components, such as histones, cfDNA, and tissue factors, activate the coagulation cascade [41]. Additionally, histones directly activate platelets via TLR2 [39] and endothelial cells (ECs) via TLR4, which leads to apoptotic EC death [42]. Platelets are confirmed as central to NET formation: through cell surface receptors P-selectin [43] and high mobility group box 1 (HMGB1) [44], albeit other, unidentified receptors await characterization, platelets adhere to neutrophils, inducing NETosis [45,46]. NET components, particularly histones, in turn, promote platelet activation. This intricate platelet-NET patho-mechanism has been implicated in generating prothrombotic states, including in COVID-19 [47].

NET enzymes, such as NE and MPO, damage ECs, leading to the exposure of von-Willebrand factor (vWF), which binds platelets. In the context of COVID-19, endothelial cell damage has been implicated as central to disease progression, with one such study considering elevated levels of vWF as the most important independent prognostic risk factor. In addition to the prothrombotic players enhanced by NETs, another mechanism by which NETs contribute to thrombophilia is inhibition of the natural anticoagulants; NET-contained serine proteases inhibit tissue factor pathway inhibitor [48] and

histones bind thrombomodulin, thereby preventing thrombin-thrombomodulin dependent activation of protein C [49].

3. Potential mechanisms underlying NET-induced cardiac disease

Although NETs are confirmed as being involved in COVID-19 immuno-thrombosis, the exact mechanisms and inflammatory dynamics culminating in their production are yet to be elucidated. In this section, we aim to provide an insight for the role of NETs in inducing COVID-19 cardiac disease; we discuss (1) direct viral cytopathic effects; (2) systemic inflammatory response syndrome (SIRS); and (3) ischemic reperfusion injury (Figure 1).

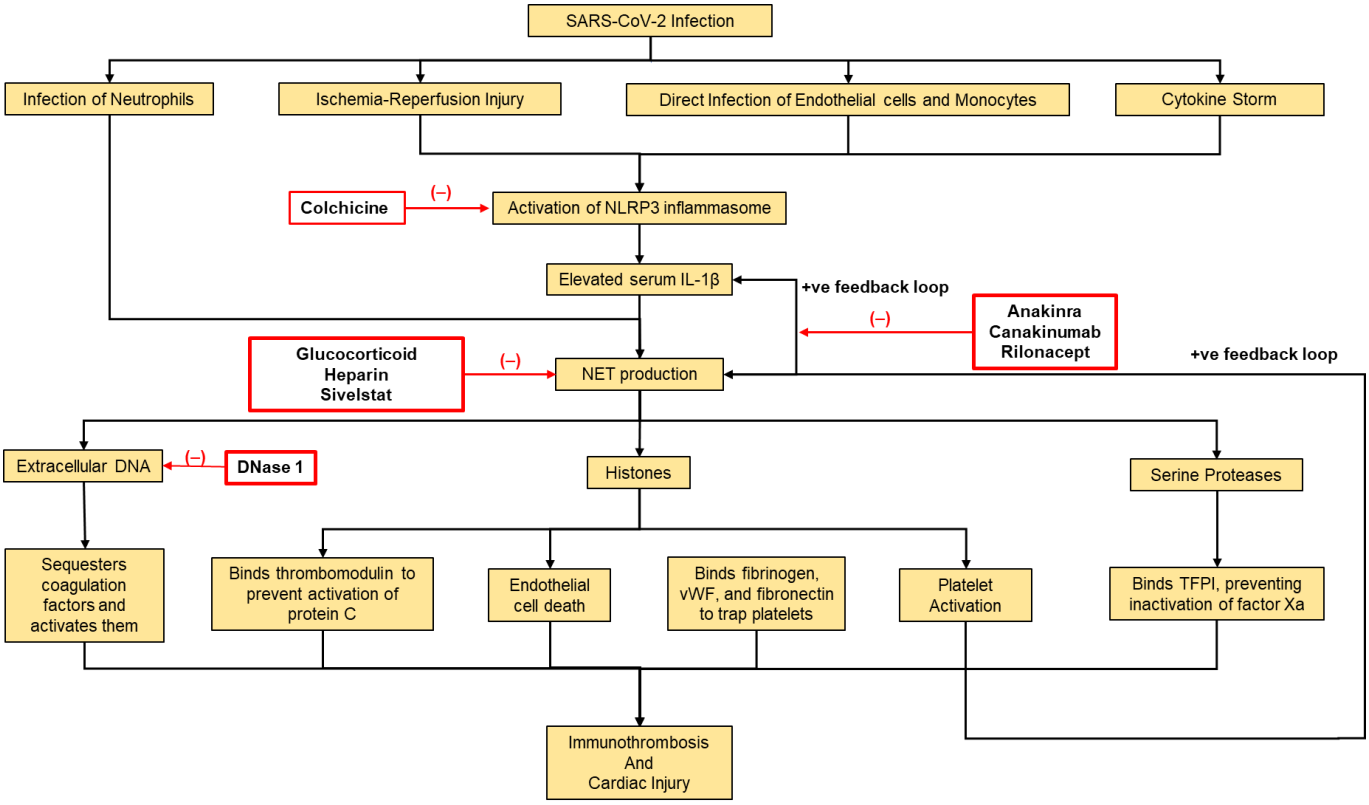

Figure 1. A schematic representation of the potential mechanisms utilized by SARS-CoV-2 in cardiac disease. Direct infection of monocytes and endothelial cells, cytokine storm or Reperfusion injury, all cause a pro-inflammatory state and activate the NLRP3 inflammasome, eventually triggering NETosis. NETs comprise various prothrombotic molecules including extracellular, cell-free DNA, histones, and serine proteases, all of which lead to an imbalance between prothrombotic and antithrombotic mediators favoring a hypercoagulable state, thus resulting in cardiac injury.

3.1. Direct infiltration

The angiotensin-converting enzyme 2 (ACE2) receptor is the portal of entry of SARS-CoV and SARS-CoV-2 into cells. Following attachment of the spike (S) protein to ACE2, cleavage of the S protein by the transmembrane serine protease 2 (TMPRSS2) allows internalization of the virus by endocytosis. The tropism of SARS-2-CoV for the heart derives from the expression of ACE2 on cardiomyocytes. This is supported by the work of Bearse et al, where SARS-CoV-2 viral particles were recognized in cardiac tissue of several COVID-19 cases by RT-PCR [50]. Moreover, Lindner et. al reported the presence of SARS-CoV-2 in postmortem myocardial tissue [51].

A study found associations between SARS-CoV-2-positive cardiomyocytes and myocarditis featuring lymphocytic and/or granulomatous characteristics, and SARS-CoV-2-positive coronary ECs and microvascular thrombosis [52]. This provides mechanistic insights into the thrombophilia induced by SARS-CoV-2, whereby infection of ECs precipitates a hypercoagulable state. Ackermann and colleagues proved the presence of intra-endothelial SARS-CoV-2 viral particles [14], implicating direct SARS-CoV-2-induced endothelitis as perhaps responsible for the distinctive angiocentric features of COVID-19. Since normal EC function is crucial in maintaining normal hemostasis, insults causing EC dysfunction to precipitate a hypercoagulable state [53]. Patients with severe COVID-19 consistently display lab parameters of hypercoagulability, including high D-dimer, increased fibrinogen, elevated prothrombin time, and activated partial thromboplastin time, and thrombocytopenia [54].

Virus-induced endothelitis leads to overproduction of proinflammatory cytokines, ROS, and acute-phase reactants. Furthermore, SARS-CoV-2 endothelitis extends systemically, manifesting with signs of vasculopathy—with direct viral cytopathic effects as a causative factor—in the lungs, brain, heart, kidney, gut, and liver. Thrombi in COVID-19 lead to disease-related multi-organ failure, featuring strokes, acute coronary syndrome, pulmonary embolism, acute respiratory distress syndrome (ARDS), deep vein thrombosis, and acute kidney injury due to widespread microvascular thrombosis [1,15].

NETs have been detected in coronary thrombi in autopsy specimens of deceased COVID-19 patients, suggesting their importance in mediating the coagulopathy of COVID-19 [55]. Enhanced neutrophil adhesion to a dysfunctional endothelium and increased interactions with platelets in these affected areas can amplify NET production. NETs in turn activate EC and induce their apoptotic death. Thus, NETs compromise endothelial barrier integrity, consequently aggravating the hypercoagulable state and leading to COVID-related organ dysfunction and/or failure. The EC compromise further propagates NETosis and hyperinflammation. Therefore, widespread pyro-ptosis of macrophages not only in the lung, but also in the heart followed by severe inflammation and hypercoagulability via direct SARS-CoV-2-induced EC and monocyte/macrophage activation, is partly responsible for the myocarditis, interstitial hyperemia, and myocardial necrosis observed in heart autopsies of deceased COVID-19 patients. This could manifest clinically as cardiomegaly and elevated cardiac biomarkers, with concomitant features of heart failure, acute coronary syndrome, and arrhythmias. Serum LDH is used as a marker of various inflammatory states and is significantly elevated in severe COVID-19 patients compared to those without the severe disease and thus can be used as a marker for severity and prognosis [56–58]. Further, it also appears that the magnitude of elevation of cardiac troponin may be associated with the severity of COVID-19 and cardiac disease with probable prognostic implications [54].

Platelets are well-known to adhere to damaged endothelium and upregulate adhesion molecules, including P-selectin and ICAM-1, which enables neutrophil-platelet interactions. Physiologically, platelets do not bind neutrophils, but activated platelets do so in the setting of microbial infection. Activated platelets adhere to neutrophils via P-selectin and HMGB1, both of which induce NETosis. NET components such as histones in turn can activate platelets. Therefore, a potential NET-platelet circuitry may contribute to propagating microvascular thrombosis, as evidenced by autopsy studies reporting the presence of microvascular thrombi comprising mainly neutrophil-platelet or NET-platelet complexes [59]. Consistently, illness severity correlates with MPO-DNA complexes (NET markers) and COVID-19 patient sera displaying elevations in NET-triggering factors.

Other than SARS-CoV-2 infection of monocytes and endothelial cells, SARS-CoV-2 directly elicits NETosis by neutrophils, like other respiratory viruses including respiratory syncytial virus and Influenza [60]. This is suggested by induction of NETosis in control-derived neutrophils by COVID-19 patient sera [28] and later proven by ACE2-mediated infection of neutrophils by SARS-CoV-2 eliciting PAD-4-dependent NETosis [30].

3.2. The cytokine storm and SIRS

Many COVID-19 patients display little to no symptoms, while others suddenly deteriorate and develop raging fevers and severe pneumonia, culminating in acute severe respiratory distress syndrome and death. Growing evidence shows that this presentation is secondary to a dysregulation of the innate immune response, resulting in a cytokine storm, systemic inflammatory response syndrome, and multi-organ failure, including the heart. Numerous proinflammatory cytokines, such as IL-1β, IL-2, IL-6, IL-10, IL-17, TNF, and monocyte chemoattractant protein 1 (MCP1) are found to be elevated in such patients [20]. Even though these are triggered by a local infection in the lungs, it is believed the increased systemic levels of these inflammatory cytokines activate inflammatory and maladaptive remodeling pathways such as NETs in multiple organs, including the heart.

A major cytokine involved in initiating and propagating the inflammatory response is IL-1β, which is activated by the NLRP3 inflammasome. Briefly, the process of inflammasome activation begins with the recognition of PAMPs or DAMPS by PRRs on macrophages, which, if activated, induces NF-κB signalling pathways, thus leading to downstream upregulation of pro- IL-1β and pro-IL-18 and assembly of the NLRP3 inflammasome [61]. Upon a second activation signal, which comprises membrane damage, ROS-induced stress, and/or activation of certain ion channels, NLRP3 components oligomerize, which forms a platform—termed the NLRP3 inflammasome—for caspase-mediated cleavage of pro-IL-1β and pro-IL-18 into active IL-1β and IL-18 [61,62]. Lastly, to release intracellular IL-1β into the extracellular space, caspase-activated gasmerdins enter the plasma membrane and oligomerize to form pores, thereby allowing IL-1β to exit. SARS-CoV-2 directly activates the NLRP3 inflammasome via its surface E protein and viroporin 3a. Indeed, inoculation of SARS-CoV-2 with macrophages induces inflammasome activation. Accordingly, the IL-1β and IL-18 are elevated in COVID-19 patients, in correlation to disease severity [63].

Furthermore, a trigger for NETs is the inflammasome-derived proinflammatory cytokine IL-1β. Serum IL-1β levels are elevated in COVID-19, with their magnitude of increase correlating with disease severity, as is also the case with NETs [64–66]. Based on this observational premise, and numerous pre-existing studies on the interplay between NETs and IL-1β, this potential circuitry has been implicated in

the immuno-thrombosis of COVID-19. In this scenario, SARS-CoV-2 infection of macrophages or endothelial cells within various organs, including the heart, activates inflammasomes, thus leading to the production of IL-1β and IL-18, which, in turn, attracts neutrophils and induce NETosis. Additionally, antimicrobial components within NETs, such as cathelicidin LL37, induce IL-1β production in macrophages after engaging their cognate surface receptors [67]. This suggests a feed-forward loop, whereby IL-1β induces NETosis, which promotes IL-1β production in macrophages [8,12].

3.3. Ischemic reperfusion (I/R) injury

Ischemic myocardial injury often results from rupture of a pre-existing atherosclerotic plaque (usually a subclinical atheroma) with consequent thrombosis and occlusion of a coronary vessel. Although classically thought to primarily involve monocytes/macrophages, mounting evidence confirms the leading role of neutrophils, which demonstrate predictive behaviors in such settings and accumulate in coronary thrombi. NETs are also implicated in sterile forms of inflammation-induced injury, including I/R injury of the myocardium [68]. In accordance, studies in animal models show that adding NET-lysing agent DNAse1 to conventional rtPA ameliorates symptoms of I/R injury, relative to rtPA and DNAse1 alone [69]. This study reported reduced NET density in coronary microthrombi, a reduced no-reflow area in the ischemic zone, and a reduced infarct size. Moreover, the adjunctive intervention demonstrated reduced I/R injury-induced LV remodeling after 45 days (about 1 and a half months), thereby improving long-term LV function [69].

From a mechanistic standpoint, ischemia causes the release of DAMPs, including histones and HMGB1, from resident cells, which activate platelets and endothelial cells to promote thrombus formation and further vessel occlusion. Reperfusion follows ischemia, encompassing a robust inflammatory response featuring dense neutrophilic infiltrates, which aggravate tissue injury. ROS are key mediators of I/R injury, potentially driving reversibly injured cells to irreversible cell damage and necrosis. In addition, ROS cause NETosis which along with histones and HMGB1-induced NETosis via TLR4 and TLR9, respectively [70], augments NETosis and consequently aggravates host tissue injury. Such models in the context of liver disease have yielded encouraging outcomes, with depletion of neutrophils, impairment of NETosis via blockade of TLR9, and/or antioxidant medications all attenuating I/R injury, thereby warranting future investigations into such an interplay in the context of COVID-19 [5].

Intriguingly, inflammasomes have been heavily studied in I/R injury. Inflammasome activation is the hallmark sensor of DAMPs released upon myocardial ischemic reperfusion injury, as indicated by the elevated expression of NLRP3 inflammasome-associated proteins in cardiac fibroblasts and cardiomyocytes. Subsequently, inflammasome-mediated production of IL-1β initiates a cascade of inflammatory responses [71]. Blockade of NLRP3 inflammasome signaling pathways via colchicine exhibits cardioprotective effects against I/R injury [72]. As mentioned earlier, IL-1β is a major inducer of NETosis, with inhibition of the latter also alleviating symptoms of I/R injury. These observations collectively show IL-1β and NETs as potentially key players in I/R injury and given the well-established interplay between the two, such an interplay in the setting of COVID-19-induced acute MI warrants further analysis.

4. NETs: potential therapeutic targets

The complex circuitry between NETs and immuno-thrombosis in COVID-19 is a potential pharmacological target to combat the untoward pulmonary and systemic manifestations of COVID-19, rationalizing clinical trials to evaluate the clinical veracity of such postulations. Until now, various medications have been utilized clinically to mitigate COVID-19 disease progression, including heparin [73], glucocorticoids such as dexamethasone [74], and hydroxychloroquine, which only recently was proven to be ineffective [75]. These drugs have demonstrated NETs-inhibiting capacities, with NETosis being less prevalent in neutrophils exposed to hydroxychloroquine in vitro, albeit admittedly an in vivo experiment has not yet been conducted [29]. Glucocorticoids, through their anti-inflammatory effects, suppress the production of NETs-inducing cytokines, whereas heparin suppresses NETs formation and accelerates their degradation [29,76], likely through their anticoagulant and anti-inflammatory effects which minimize potential interactions between neutrophils and platelets. Consistent with our postulation is the observed benefit of heparin administration in COVID-19 coagulopathy, although certain patients develop resistance to heparin. Additionally, numerous studies have indicated heparin as not reducing mortality in COVID-19 patients, although moderately ill patients reported improving survival. Bleeding diathesis is a major concern with heparin use, with trials showing an increased risk of major bleeding with heparin than with thromboprophylaxis [72].

Another intriguing option is clearing the already formed NETs via multiple hypothesized treatment strategies [77]. One such promising avenue is NET lysis via DNAse1 (Dornase alfa). DNase mediates NET lysis, thereby combatting immune-thrombosis characteristic of COVID-19 [78]. A study analyzing the clinical data and outcomes of five mechanically ventilated patients consistently showed a reduction in supplemental oxygen requirements in all five patients [79]. However, it is important to note that the DNase administered in this study was not given intravascularly but via an aerosolized mist; there is a paucity of literature looking at the intravascular DNase administration due to no pharmaceutical agent yet registered for this purpose. Furthermore, concluding such a small study would be rather speculative. Nevertheless, DNase also enhances the digestion of retrieved thrombi from ischemic stroke patients when added to conventional tissue plasminogen activator (tPA) therapy [80], and similar results were noted in the context of myocardial I/R injury, confirming the existence of NETs in coronary microthrombi [4]. Additionally, DNAase I, as well as DNase 1L3 maybe therapeutic in helping treat digital necrosis of the extremities, secondary to potential NET formation in blood vessels; this is a severe, rare complication of COVID-19 and can contribute to mortality of patients. Hence, future clinical trials are of prime importance for determining utility of this pharmacotherapy in critically ill COVID patients [81]. In short, since systemic hypercoagulability is a salient feature of severe COVID-19, DNase together with tPA could constitute a valid therapeutic option [78]. To date, a total of seven studies are currently investigating the use of Dornase alfa in COVID-19 patients [82].

Three studies are currently looking at the therapeutic benefit of statins in COVID-19 patients, given its multiple effects on influencing the reduction of inflammatory markers, inhibiting thrombogenicity, and improving endothelial function [83]. Further, the mechanism hypothesized towards thrombosis and digital necrosis in COVID-19 patients may be similar to the patho-mechanism underlying coronary thrombi, particularly considering the possible role of NETs. Drugs like Lactoferrin can theoretically block NET production, and many trials evaluating the clinical efficacy of this protein in COVID-19 are currently being carried out [84]. In addition, Dipyridamole acts as an agonist at the (A2A) adenosine

receptor, reducing the release of NETs by neutrophils. A small clinical study from China encourages the prophylactic use of dipyridamole in COVID-19 patients [85,86] and three registered clinical trials evaluating the same are underway.

Other drugs target key proteins in NETosis, such as NE and PAD4: NE inhibitors such as sivelestat are indicated for the treatment of ARDS in Asia since 1998, although no significant improvements in survival rates have been observed [75]. Cl-amidine prevents NETosis by irreversibly inhibiting PAD4, thereby blocking chromatin decondensation, and was developed for severe inflammatory diseases where NETs are key players, and such is the case with COVID-19 [82].

Alternatively, other than inhibiting NET production or enhancing NET lysis, exploiting the amplification circuitry between IL-1β-NETs circuitry also represents a potential therapeutic route. Anakinra is one such drug that antagonizes the IL-1β receptor and reduces NETs in various inflammatory conditions, based on which we previously advocated for its use in COVID-19 [8]. Encouragingly, a recent study evaluating the efficacy of early anakinra administration in 528 patients with an elevated risk of progressing into severe disease reported a significant reduction in progression to severe disease as well as reducing severe disease [87]. Other IL-1 receptors antagonist canakinumab and rilonacept are currently being evaluated for use in COVID-19 [87]. Lastly, colchicine has shown cardioprotective effects in acute myocardial infarction, due to inhibiting the NLRP3 inflammasome, which plays a role in ischemic reperfusion injury. All the drugs mentioned are indicated in Figure 1.

As concluded by Barnes et al., NET-targeted therapies should be considered in the management of COVID-19 to reduce the severe systemic inflammatory response and overall mortality [88]. However, given the essential role of NETs in host defense, the benefit of inhibiting their production must be counterbalanced against the deleterious effects NET inhibition could have on disease progression and thereby patient trajectory. Also, the multifaceted immunopathology caused by SARS-CoV-2, along with the differential responses shown by patients suffering from varying severities, is suggestive of a severity-grade dependent dysregulation of the immune response. Therefore, it is plausible that certain patient populations may benefit from NET inhibition, while it worsens outcomes in other patients. Indeed, such immunosuppressant effects during the initial stages of disease could potentiate disease progression, whereas suppressing the dysregulated immune response could improve the condition of more critically ill patients; these effects have been reported for other therapeutic modalities, including mesenchymal stem cell use [89].

5. Conclusions and future directions

Mounting evidence implicates cardiac disease being a salient feature of severe COVID infection. Further, myocardial injury is significantly associated with more fatal outcomes [4]. However, the mechanisms underlying such presentations are currently uncertain. We reviewed the potentially key role played by NETs in COVID-19 myocardial injury, in hopes that this will instigate future studies testing the veracity of such postulations. If proven favorable, then potentially novel therapeutic strategies could be employed to combat COVID-19 pathogenesis.

Attempts to quantify NET presence against other blood clot components are currently lacking. These studies would also indicate how efficacious attempts to block NETosis or enhance NET degradation would be in ameliorating COVID-19 pathogenesis. Previously discussed murine studies show that adding NET-degrading agents such as DNAse1 to conventional rtPA therapy significantly

improves outcomes of acute MI (Myocardial Infarction) with ischemic reperfusion injury [68]. Comparable results were obtained by another study reporting a significant enhancement in thrombolysis of retrieved ischemic stroke thrombi from patients [78], which is also a disease manifestation of severe COVID-19 [90]. Therefore, conducting such in vitro analyses in the context of COVID-19 could further our understanding of the major patho-mechanisms utilized by SARS-CoV-2, while concurrently perhaps also furthering discussion on prothrombotic disorders regardless of etiology. Lastly, it is prudent to scrutinize the degree of NET involvement in COVID-19 thrombi, i.e., whether NETs play a major or minor role in COVID-19 hypercoagulability. Already existing studies are encouraging in this regard, demonstrating rich NETs infiltration in coronary microthrombi [54].

Conflict of interest

The authors declare no conflict of interest.

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